Radiation-induced Sterility in the Pink Bollworm

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List of Chemicals Mentioned in this Publication

1. Azinphosmethyl: *O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazon-3(4H)-yl)methyl] phosphorodithioate

2. Carbaryl: 1-napthyl methylcarbamate

3. DDT: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane

4. ENT 50761: bis(1-aziridinyl)phosphinic acid ethyl ester

5. Malathion: diethyl mercaptosuccinate S-ester with *O,O* dimethyl phosphorodithioate

6. Methyl parathion: *O,O*-dimethyl *O*-(*p*-nitrophenyl)phosphorothioate

7. Monocrotophos: dimethyl phosphate ester with (*E*)-3-hydroxy-*N*-methyl-crotonamide

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ABSTRACT

The pink bollworm is a primary pest of cotton in the southwestern United States and many other parts of the world. As such, it has received much attention as a candidate for the Sterile-Insect-Release-Method (SIRM) of control. Literature on radiation biology of the pink bollworm covers a timespan of over 15 years and many publication sites. This review summarizes the results of that research and makes recommendations on further avenues of work that should be carried out to help scientists and program managers make informed decisions on treatment regimens for maximum effect in a SIRM program.

KEYWORDS: pink bollworm, radiation induced sterility, delayed sterility, radiation biology, SIRM, sterile insect release method

CONTENTS

RADIATION-INDUCED STERILITY IN THE PINK BOLLWORM

By Alan C. Bartlett[1]

INTRODUCTION

The successful use of radiation-induced sterility to eradicate the screwworm fly from the southeastern United States (*6*)[2] led to the initiation of radiation studies on the pink bollworm in 1961 (*32*). Since that time, numerous researchers have studied the radiation biology of the pink bollworm in an attempt to determine optimum treatment conditions for the production of competitive sterile individuals. No comprehensive review or summary of this literature has been published even though a sterile insect release program has been conducted since 1969 in the San Joaquin Valley of California. The present paper is an attempt to correlate this data, to explain some apparently contradictory results, and to suggest efficacious treatment levels and conditions that can be used by scientists for further research or by program officials for informed decisions.

No attempt will be made in this paper to review the theory of the Sterile-Insect-Release-Method (SIRM) of control. A good review of the method is found in Davidson (*9*). LaChance (*22*, *23*) reviewed problems related to control of lepidopterous insects and the status of SIRM on the world scene.

DEFINITION OF TERMS

Since several different measurement units of radiation are used in the literature, it seems important to try to quantify the units for a better understanding of the doses applied to the pink bollworm. The unit most commonly used in early literature is the roentgen (R). One roentgen is the quantity of X- or gamma radiation such that the associated corpuscular emission per 0.001293 gram of air produces, in air, ions carrying 1 electrostatic unit of electricity of either sign. When higher doses of radiation are applied, as in most insect studies, the term used to describe dosage is usually "kiloroentgen" (kR), which indicates 1,000 R.

Authors of later literature commonly used the term "rad," which is an acronym for radiation-absorbed-dose. A rad is 100 ergs of absorbed energy per gram

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[2]Italic numbers in parentheses refer to Literature Cited, p. 22.

of absorbing material (that is, soft tissue). Thus, the rad is a measure of energy absorbed by the irradiated material. For X-rays and gamma rays, 1 rad = 1 R[3]. The unit is usually expressed in entomological work as krad (1,000 rad). The term "krad" will be used in this paper to describe radiation treatments. Dosimeters can be purchased that read dosage in either roentgens or rads, depending on the system used and calibration procedures.

A number of other terms are used in the literature that are clear to workers in the field, but may be confusing to others. An attempt is made here to define some of these terms since they will be used in the literature review.

Eupyrene and Apyrene Sperm

The pink bollworm and other lepidopterous males produce two kinds of sperm during spermatogenesis (especially during the last stages of spermatogenesis, at pupation and during the pupal period). The apyrene type of sperm results from a degenerative type of meiosis in which the nuclear material (the chromatin) seems to be lost or to degenerate (*16*, *45*). The eupyrene type of sperm is formed during normal meiosis and has nuclear material in the proper location in the sperm head. In the pink bollworm, eupyrene sperm remain in bundles until transferred to the female during copulation, whereas apyrene sperm bundles break down before they reach the duplex region of the male reproductive tract (*26*). Eupyrene sperm fertilize the eggs. The exact function of apyrene sperm is not fully understood, but they may help to transfer eupyrene sperm from the bursa copulatrix of the female to the spermatheca for storage. LaChance et al. (*26*) found that normal amounts of eupyrene sperm in the spermatheca of a mated female were necessary to elicit normal egg production. The presence of eupyrene sperm in the spermatheca of a female is used in several of the papers as a criterion of normal mating by treated and untreated males.

Identification Key

In certain sections of this paper, it is necessary to distinguish among generations of insects, different types of treatments, and different strains of insects. The following key should be helpful in identifying various combinations of factors:

N or native = pink bollworms collected from cottonfields either as larvae or pupae.

L or laboratory = pink bollworms reared in the laboratory on artificial diet for more than one generation.

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[3]An X-ray or gamma ray dose of 1 R gives an absorbed dose of 93 ergs per gram of tissue, but the difference between 93 and 100 ergs is generally ignored in the literature. The rad has supplanted the rep, which was a unit representing the absorbed dose physically equivalent to 1 roentgen of X-radiation.

U or untreated = control group or mating of pink bollworms that have been handled in every way similar to treated groups except for irradiation (or other treatment).

T or treated = treated group, may be one of several treatments under study as designated.

R_0 or irradiated = treated generation of a series of generations under study--one or both parents could have been irradiated.

R_1 = first generation of progeny from irradiated parent or parents.

R_2 to R_n = second and subsequent generations of progeny from parents irradiated in the R_0 generation.

P_0 or parental = untreated or control generation of a series of generations under study.

F_1 = first generation of progeny from untreated parents.

F_2 to F_n = second and subsequent generations of progeny from untreated parents.

Somatic and Genetic Damage

When an individual is exposed to ionizing radiation, two types of cellular damage may be sustained. If the radiation is absorbed by any generalized or specialized cell of the body, except for the germ (reproductive) cells, that cell can experience either cytoplasmic or genetic damage. Such damage is not passed to later generations of progeny. In this paper, such damage is called somatic damage. An example of this type of damage is that experienced by a pupa exposed to a dose of gamma radiation. The radiation can produce morphological or developmental abnormalities in that exposed individual so that it cannot subsequently reproduce or even eclose as a normal adult. However, such damage is not passed on to subsequent generations.

In contrast, an exposed individual can sustain genetic damage, which is passed on to subsequent generations. Genetic damage can result whenever the nuclei of reproductive cells of the organism are exposed to radiation treatment. Genetic damage may or may not be associated with observable somatic damage in a treated organism. In fact, an ideal radiation exposure would produce minimum somatic damage with maximum genetic effect. The genetic material would be damaged, but reproductive functions, physiological functions, and morphological functions of the insect would be intact. The common experience of investigators is that doses of radiation that maximize sterility also produce significant physiological or reproductive dysfunctions. The more immature the stage of the insect at treatment, the more critical becomes the factor of somatic damage.

Cut-out Stage

A term frequently used in pink bollworm research to refer to a stage of larval development at which the mature larva leaves the vicinity of the food and forms the prepupal stage of development is the "cut-out stage." Under natural field conditions, the larva chews its way out of the boll and falls to the ground

to pupate. Under many laboratory rearing conditions, the larva chews its way out of the rearing container (usually a waxed cardboard cup or tub) and falls into a suitable pupation site (Hexcell, vermiculite, cardboard strips, and so forth). Larvae at this stage are called cut-out larvae. The cut-out stage offers a convenient means for separating the larvae from their rearing medium and concentrating them for further steps in rearing or treatment.

CRITERIA FOR RELEASED STERILE INSECTS

Sterile insects must be similar to native insects in important aspects of reproductive behavior and physiology. Released males must be able to search out and find native females. They must be as sexually attractive to the female as a natural male so that mating will be on a competitive basis. Released males must have the same or closely similar diurnal rhythm as native males. The released male must also be able to successfully transfer sperm to the female so that her physiological state is that of a successfully mated female. The released female must be able to emit pheromone attractive to the native male (as well as to the released male if releases of both sexes are carried out simultaneously) and released female diurnal rhythm must closely match that of the native female. The released female must be willing to submit to insemination by native males, and she must not produce any fertile progeny (preferably, she should not produce any viable eggs).

Typically, insects which have been laboratory reared and sterilized with radiation do not meet all or even most of these criteria. Many researchers have found that sterilizing doses of radiation produce males that are not sexually competitive or do not successfully transfer sperm. As a result, the female mated to a sterile male does not act like a successfully mated native female. For example, the egg production of untreated pink bollworm females mated with irradiated males is markedly reduced (*32*), and such females also mate more frequently than do females mated to untreated males (*8*).

If the competitive ability of the released insects is lowered by treatment (either rearing, irradiation, or subsequent handling), then this reduction must be compensated for by increasing the numbers of released insects, thus increasing the costs of the release program.

LITERATURE SURVEY

Egg Stage

Two studies have been conducted in which investigators examined the fertility of adult pink bollworms that were reared from irradiated eggs.

Wolfenbarger and Mangum (*44*) irradiated eggs at 1, 2, 3, and 4 days after oviposition, using doses of 1, 2, 4, and 8 krad gamma radiation. The dose of 8 krad given to any age of eggs essentially eliminated egg hatch. All doses appeared to reduce the number of viable adults developing from the eggs; however, since no attempt was made to record percentage of egg hatch of the irradiated eggs, no conclusions were reached concerning the susceptibility of different ages of eggs to dose of radiation. Adults that developed from irradiated eggs

were mated to untreated adults and checked for fertility. The observed criterion, egg hatch, was extremely variable and no clear-cut trends were observed; however, doses of 1 and 4 krads produced some statistically lower egg hatch from both males and females.

Bartlett et al. (*4*) irradiated 4-day-old eggs with doses of cobalt-60 gamma radiation ranging from 2 to 32 krad (doses = 2, 3, 4, 6, 8, 12, 16, 32 krad). Four-day-old pink bollworm eggs, under the conditions of these studies, were completely developed, and about 10 percent had hatched at the time of irradiation. None of the doses of radiation inhibited hatch of the eggs when compared with the control. As dosage was increased over 2 krad, development of larvae from these irradiated eggs showed a significant decline but development time increased. The numbers of adults obtained from eggs treated with more than 8 krad were reduced about 0.1 percent of the control, and it took 2 to 3 days longer for these moths to emerge.

Adults from each of the doses were mated among themselves to check for fertility. Egg production was reduced at all doses (ranging from a 17-percent reduction at 2 krad to over 99-percent reduction at all doses over 4 krad). Percentage hatch of these eggs ranged from 58 percent in the control down to 17 percent at 4 krad. Females from all dosages were checked for mating and sperm transfer with the results showing that all doses, both males and females were reproductively inferior to normal adults. Even at the 2-krad treatment, 67 percent of treated males did not mate. Only those individuals treated as eggs with 2 krad showed normal rates of emergence, but at this dose, sterility was not complete.

Both of these studies of egg irradiation indicated a poor prognosis for egg treatment for inducing adult sterility along with good competitive ability. Neither of these studies investigated the effects of egg irradiation on the fertility of R_1 progeny from matings of the treated individuals with untreated adults. Lepidoptera can exhibit a pheonmenon referred to as inherited sterility, F_1(= R_1) sterility, or delayed sterility. This phenomenon will be discussed in a later section of this paper. Thus, further studies on egg irradiation could be fruitful if treatments do not exceed about 2.5 krad and if R_1 progeny from the treated individuals exhibit inherited sterility. Such studies should be carried out to complete this aspect of pink bollworm radiation biology.

Larval Stage

Only one study has been published on the effects of treating pink bollworm larvae with radiation to induce sterility. Bartlett and Lewis (*3*) irradiated last instar (cut-out) larvae at doses of 2 to 32 krad of cobalt-60 gamma radiation (doses = 2, 4, 5, 8, 10, 15, 16, 20, and 32 krad).

Samples of the irradiated male larvae were dissected and checked for chromosomal damage. Forty percent of the treated male larvae showed chromosomal damage at the lowest dose used (2 krad). At 5 krad or higher, 100 percent of the examined spermatogonial divisions showed gross chromosomal damage. None of the doses tested had a significant effect on pupation of the treated larvae, but the percentage of morphologically normal adults was reduced by more than 85 percent when doses exceeded 4 krad for the males or 2 krad to the females.

No reproduction occurred in any crosses involving treated insects if the dose to either mate exceeded 5 krad. If the female of a cross was treated, no reproduction occurred at any dose exceeding 2 krad. When adult males from treated larvae were mated to untreated females, there was no reduction in progeny numbers at 2 krad; at 4 krad, progeny numbers were decreased by 27.6 percent; and at 5 krad, the reduction was 53.5 percent of the control.

Mature R_1 progeny from treatments of 2, 4, and 5 krad were mated in all possible combinations of treated and untreated mates to observe the reproductive ability of these progeny. (See section on "Inherited Sterility" for theoretical discussion of these tests.) The R_1 females had low fecundity (values of egg production ranged from 0 to 30 percent of control) and fertility (numbers of mature R_2 progeny ranged from 0 to 24 percent of control values) regardless of dosage received by their parents. R_1 males crossed to R_1 females produced less than 0.5 percent of the number of progeny produced by normal controls. R_1 males crossed to untreated females produced between 0 and 19 percent of the control progeny. Most of this reduction was due to decreased egg production by the females that had mated to these R_1 males. No differences from control matings could be shown for the number of matings or the number of males transferring sperm.

This study indicates that further experimentation could be done on larval irradiation, especially at doses of from 2 to 4 krad. Such tests must include competition tests to evaluate the competitive ability of the adults emerging after larval treatment.

A field-cage test on the competitiveness of adults emerging from larvae irradiated at 2 and 4 krad cobalt-60 gamma radiation (Bartlett, unpublished report) gave results showing that a 1 treated:1 normal release would not produce an observable population decline. However, the variability of replications in these small field cages was large enough to mask any real effect. Such competition studies might be carried out under laboratory conditions to see whether control of a normal population can be achieved by such treatment or in field cages using greater ratios of treated males.

Larval treatment has some appeal in a mass release program for the pink bollworm because the cut-out larvae are easily collected and handled under present mass-rearing conditions. A modification of the cobalt-60 irradiator to handle large sheets of pupation material would be the only significant change necessary. After irradiation, larvae could be held for emergence at the rearing site and shipped as adults, or the treated larvae could be shipped to a release point and allowed to emerge on site. Emerging adults could be released by airplane, or they could be released at ground level from appropriate emergence boxes.

Two unpublished investigations were carried out in the winter of 1975 and spring of 1976 to test the effects of gamma irradiation on emergence of larvae from diapause. Bartlett (unpublished report) treated diapausing larvae with doses of 3, 5, 7, 9, and 11 krad. Analysis of emergence rates of control and treated larvae held under insectary conditions showed that doses of 3, 5, and 7 krad gave rates of emergence not statistically different from the control emergence rate. However, at 7, 9, and 11 krad, significantly fewer adults emerged than at 0, 3, and 5 krad. At 9 and 11 krad, the emergence rates were significantly delayed as compared with the control rate.

R. T. Staten and A. C. Bartlett (unpublished data) repeated the same test under field conditions using pyramidal emergence cages. Again, doses of 3 and 5

krad emerged at rates similar to the control, but doses of 7, 9, and 11 krad emerged significantly slower and in lower numbers than the control.

The results of these tests indicate that diapausing larvae can be treated with irradiation and be expected to emerge normally under field environmental conditions. Fertility tests on adults emerging from such treatments must be conducted to see whether the response is similar to that seen when nondiapausing larvae are irradiated.[4] Development of this technique could lead to the successful evaluation of overwintering capabilities of pink bollworms in cotton-producing areas where they do not now occur (such as the San Joaquin Valley of California).

Pupal Stage

The first reported pink bollworm radiation tests, by Ouye et al. (*32*), were carried out using pupae. These tests revealed that pupal age (time after pupation) significantly affected radiation sensitivity. The younger the pupa was at time of radiation, the more somatic, physiological, and genetic damage was sustained by the pupa. The results of treating 1- and 3-day-old pupae were very similar in that increasing doses of radiation resulted in increasing amounts of pupal mortality and adult malformation. Six krad was sufficient to eliminate over 90 percent of all treated pupae at 1 day; however, it took 14 krad at 3 days of age to achieve this amount of damage. At 5 and 7 days of age, pupal mortality and/or adult damage were insignificant even at very high doses of radiation. The authors concluded that 7-day-old pupae (which were within 1 day of eclosion at 29.4° Celsius) were at the best pupal age to irradiate. In this study, Ouye et al. (*32*) used egg hatch as the criterion for sterility. They concluded that 7-day-old male pupae required over 50 krad for complete inhibition of egg hatch if the treated males were mated to an untreated female. Females treated as pupae with doses over 35 krad gave no egg hatch and, indeed, if treated with over 45 krad, produced only a very few (infertile) eggs. The authors showed that egg production per female was greatly reduced if treated males were mated with untreated females, being only 37 percent of control at the lowest dose (25 krad) tested.

Longevity of male pink bollworm adults that had been treated as pupae was reduced at all doses tested by Ouye et al. (*32*). At the lowest dose tested (35 krad), survival was 17.1 days compared with 24.4 days in the control. The authors concluded that this should not have a significant effect on a release program since most mating takes place well within a 17-day lifespan.

J. C. Keller (1966, unpublished data) released moths irradiated as mature pupae with 10 krad of gamma radiation into 1-acre cotton plots in the vicinity of Humbolt and Rim Rock, Ariz. The irradiated moths were released at a rate of 20 treated:1 normal. Other field plots were used as controls with untreated adults released at the same time as in the treated plots. In an estimated five generations, the controls increased at a rate of roughly 3.3-fold per generation. The

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[4]Proshold and North (*33*) concluded that irradiated diapausing *Heliothis virescens* pupae could not be used to obtain sterile insects for release programs. At the dosages of gamma radiation they used, pupal survival was seriously reduced, but surviving pupae produced fertile adults and the R_1 progeny were also fertile.

treated plots increased an average of 1.2-fold per generation or roughly a 64-percent reduction in population growth rate over the season.

An evaluation of irradiation of pupae compared with irradiation of adults will be made at the end of the next section; however, the point can be made that only mature pupae within 24 hours of eclosion are good candidates for radiation treatment. Irradiation of earlier ages of pupae produces gross morphological abnormalities in the resulting adults. This is to be expected since the pupal stage is physiologically very dynamic and thus very sensitive to radiation damage.

Adult Stage

Most of the reported pink bollworm radiation work has been done on the adult stage. Several researchers at the SEA-FR laboratory in Brownsville, Tex. (including M. Ouye, H. Graham, D. Wolfenbarger, and R. Garcia), conducted numerous laboratory tests on the doses of radiation necessary to sterilize adult pink bollworms. A great deal of this developmental work is only found in the quarterly reports of the Brownsville laboratory.

M. Ouye (unpublished data) reported that the sterilizing dose for males was 40 krad of gamma radiation, whereas females could be completely sterilized with 30 krad. In both tests, treated insects were mated to untreated mates. Longevity and mating of both sexes appeared normal at these high doses of radiation. Egg production decreased at all doses compared with that of the control. Ouye also observed that those larvae that did survive after one of the parents was irradiated showed a high degree of sterility as adults.

As a result of these preliminary tests, Richmond and Graham (*35*) reported the results of field cage tests of the ability of newly emerged irradiated pink bollworms to suppress a normally developing population. One release of moths at a ratio of 50 treated:1 normal, treated as adults with either 25 or 40 krad, reduced the first- and second-generation populations by 72 to 95 percent of the population that developed in control cages. There was no significant difference between doses although 25 krad appeared to give better control than 40 krad. These authors concluded that 25 krad should be the preferred dose in any field trials of the SIRM on pink bollworm. They also concluded that SIRM should be maintained through the season to achieve suppression.

Richmond and Graham (*36*), in a followup of their previous field cage tests, released adult moths irradiated at less than 1 day of age with 15 or 25 krad gamma radiation into field cages at a ratio of 25 treated:1 normal. Releases were made on a continuous basis every 4 days during the first and second generations in the field cages. The released treated insects produced significant reductions in population size compared with the insects in the control cages; however, there were no significant differences in population size between the two doses. Neither treatment completely controlled the growth of pink bollworms in the field cages since some population expansion did occur in all treatments; however, as the number of pink bollworms in check plots increased 5.6 times in the first generation, those in the treated plots increased only 1.8 times (15 krad) or 1.7 times (25 krad). By the end of the experiment, the number of pink bollworms in check plots had increased by approximately 12.2 times, whereas those in the treated plots had increased 2.3 times (15 krad) and 1.8 times (25 krad).

Graham et al. (*18*) reported several laboratory experiments designed to show which doses of radiation would induce either full or inherited sterility in the pink bollworm. A complete discussion of their results will be given in a later section on inherited sterility.

In these experiments, the doses given to a 1-day-old adult male pink bollworms ranged from 5 krad to 40 krad. Egg production and hatch were reduced at every dose. Egg production was reduced 13 percent at 5 krad and up to 77 percent at 40 krad. This reduction in egg laying by a female that had received no irradidiation is typical of other species of Lepidoptera under radiation treatment. Egg hatch ranged from 69 percent, when the male parent was treated with 5 krad, down to 3.9 percent at 40 krad. This can be compared with 82 to 85 percent hatch in untreated matings. Progeny numbers are only reported for doses to males from 5 krad to 20 krad, but progeny numbers were reduced from 43 percent (5 krad) to 98 percent (20 krad) as compared with the results obtained from the control.

Graham et al. (*18*) also reported the effects of radiation on female pink bollworms mated with untreated males or with males treated at the same dose of radiation. Females were affected much more severely by the direct (somatic) effects of radiation than males. Egg production (a somatic effect) and egg hatch (a genetic effect) were severely curtailed at 20 krad and effectively eliminated at 30 krad. Progeny numbers, resulting from matings of irradiated females to untreated males, were reduced by 89 percent at 10 krad and 95 percent at 15 krad. If both the male and female parents were irradiated with the same dose, progeny numbers were reduced by 78 percent at 5 krad and 97 percent at 10 krad. No reproduction occurred if both parents received 15 krad or more. According to these investigators, irradiation had the same effect on females whether it took place before or after mating.

One further interesting fact reported in this paper (*18*) is that the proportion of males in the progeny of matings between treated males and untreated females increased with increasing dose. The proportions ranged from 1 male:1 female in the control up to 4.6 males:1 female at 20 krad. This change in sex ratio was not observed if females only were irradiated.

Bariola et al. (*2*) released moths that had been treated with 10 krad of gamma radiation into field cages. Adults were released into cages at ratios of 20 treated:1 normal and 50 treated:1 normal for the initial infestation of the cages. Seven weekly releases of 200 treated pairs were made in the 20:1 cages, whereas 500 treated pairs were released in the 50:1 cages. Since normally expanding populations were not established in any of the field cages, no conclusion could be reached concerning the released insects. The test did establish that damaged chromosomes could be passed from irradiated insects to their progeny at the dose (10 krad) used. The second important observation in this paper was that release of large numbers of partially sterile insects did not lead to destructive infestation levels of pink bollworms. The authors also concluded, on the basis of recapture studies, that releases must be made more than once a week to be effective.

Flint et al. (*11*) considered the contribution of released treated females to a suppression program. Treated females must be able to attract males for mating for a period of time similar to that of native females, but the treated females should not contribute progeny to the field population unless those progeny are themselves sterile. Thus, Flint et al. (*11*) irradiated adult females with doses of 10 and 25 krad and evaluated the attractiveness of these females

to native males. In three tests at different locations, they could find no difference in the ability of treated females to attract males as compared with untreated females of the same laboratory strain. Dosage similarly had no effect on attractiveness of the treated females.

Since investigators suspect that laboratory-reared insects might act differently than native insects, Flint et al. (*11*) also compared mass-reared (laboratory) moths with field moths and found that the mass-reared females were as attractive as native females.

Flint et al. (*11*) found that mass-reared females were not affected by radiation if the measured variable was mating ability, but the ability of these same females to transfer sperm from the spermatophore to the spermatheca declined as the dose of radiation increased. Their data indicate that a decline in ability to transfer sperm took place at the lowest dose given (5 krad), but a significant difference occurred only at doses over 15 krad. No statistically significant effect of radiation dose up to 25 krad could be shown for the lifespan of treated females.

Flint et al. (*12*, *13*) released mass-reared irradiated adults into small field cages to test the ability of such treated individuals to control a population of native pink bollworms developing in the cage. The first test (*12*) compared radiation doses of 10 and 20 krad and results indicated that treatment of 10 krad was more effective in suppressing a native population than 20 krad, although both doses had an effect on population growth in these cages. The second test (*13*) compared 10 krad-treated moths with moths that received a chemical sterilizing treatment (bis(1-aziridinyl) phosphinic acid, ethyl ester). Again the 10-krad treatment was the most effective treatment. In fact, the releases of chemosterilized moths had no measurable effect on the developing pink bollworm populations.

R. T. Staten (unpublished data) released moths irradiated with 10 krad into small fields of cotton (3.5 to 4.5 acres) in the Moapa Valley of Nevada. These tests were conducted in 1971 and 1972. In 1971, only two definite conclusions could be drawn from the experiment: (1) The released irradiated moths reproduced in the field and produced larvae that showed chromosomal aberrations; (2) immigrations of native moths took place from infested fields 100 miles or more away from the test plots. In 1972, the results were more clearly defined because adequate populations developed in the nonrelease fields to show differences between the treated and nontreated populations. Again, chromosomal abnormalities were observed in larvae from fields where treated adults had been released. Significantly more adults were captured in nontreated fields as compared with those captured in treated fields. Release ratios were apparently not sufficient to cause eradication of the population, but suppression was demonstrated.

Flint et al. (*14*) examined dispersal and mating of irradiated (20 krad) laboratory pink bollworms after release in cottonfields. They used radioactive phosphorous as the marker since the radioactivity enabled them to find the moths after release. They found that the released males remained predominantly within 120 meters of the release point. No effect of the radiation treatment on the mating ability or dispersion of the treated male could be demonstrated.

LaChance et al. (*26*) examined mating, sperm transfer, and oviposition of radiation-treated and untreated laboratory moths and compared them with native moths. These authors reached a number of interesting conclusions concerning the treated (20 and 30 krad) laboratory moths. First, they concluded that laboratory

females (paired with treated or untreated males) mated more often than did native females under laboratory conditions. Ovipositional response of laboratory-reared females was also greater than that of native females. Reduced oviposition was observed in untreated females mated to irradiated males (as compared with matings of unirradiated males and females). Treated males caused a lowering of egg production in their unirradiated mates as compared with matings of unirradiated males and females. Ability of males to transfer sperm was not affected by radiation, but native males transferred normal amounts of eupyrene sperm more often than laboratory males. Thus, some differences were shown to exist between laboratory-reared insects and native insects, but the differences did not affect the ability of treated insects to mate and to interfere with the reproduction of field populations.

Flint et al. (*15*) irradiated groups of 1,500 mixed sex adults with 10, 12.5, 15, or 17.5 krad of cobalt-60 gamma radiation to examine reproduction from large numbers of treated pink bollworms. These authors postulated that treatment of large numbers of adults would give a more realistic estimate of progeny production than that seen in previous investigations. As expected, progeny production decreased with increasing dose. The number of R_1 adults produced from each treatment ranged from 3,531 in the control to 3 at 17.5 krad. Up to one-third of the resulting R_1 adults were deformed as compared with 2 percent deformed in the control. Sex ratios of the R_1 adults were distorted with an excess of males, as seen in other studies. R_1 X R_1 matings were sterile at the 15- and 17.5-krad treatments and partially sterile at 10- and 12.5-krad treatments. The 10-krad treatment was less than 5 percent of control egg production.

Flint et al. (*15*) concluded that although a dose of 17.5 krad did produce highly sterile pink bollworms, there was a chance that some R_1 larvae could be found in fields subjected to mass releases of treated insects. The presence of these R_1 larvae could lead to problems in survey and monitoring plans. Therefore, Flint et al. (*15*) concluded that doses of radiation exceeding 17.5 krad should be used in programs where no R_1 infestation is tolerable.

At this point, we should evaluate irradiation at the pupal stage as opposed to irradiation at the adult stage. The literature indicates that as long as the pupae are fully mature, few individuals are lost due to somatic damage, and genetic damage appears equivalent at the two stages. A choice between treatment of adults versus mature pupae would seem to rest on the ability of investigators to handle the two stages.

The pupal stage may be easier to handle since one does not have to worry about mobility of the insects at that stage. The pupae do not have to be anesthetized or cooled[5] for treatment or shipping; however, the experience of most rearing facilities has shown that so much variation in physiological age occurs even in a 24-hour larval collection period that some pupae will be quite young as others are emerging as adults. In other words, it has been difficult, under presently used rearing procedures, to obtain a uniform age group of pupae for treatment.

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[5]A problem observed in some other insect release programs is the dissipation of metabolic heat from shipping containers. Thus, some cooling may be needed for shipping even for the pupal stage.

At present, adults are allowed to move on their own to a collection chamber and are then cooled and concentrated into cartons for irradiation. There are at least two undesirable features of this system. First, and probably most detrimental to the insects, is the lack of feeding facilities in the first several hours (or perhaps several days) of their adult life. A second problem is the loss of scales. This factor creates a hazard to the handling personnel and also produces an insect that may be less capable of sustained flight and more subject to environmental stress than a fully scaled insect.

If present mass-rearing procedures could be modified to produce uniformly aged pupae, then irradiation of this stage would appear to have desirable features for handling and shipping treated insects. However, careful evaluation of the ability of irradiated male pupae to compete and transfer sperm in competition with native males would still need to be done.

Inherited Sterility

Investigators studying several lepidopterous pests demonstrated that progeny raised from parents that had been partially sterilized with a radiation treatment were themselves partially or fully sterile (*31*, *34*, *42*). As a result of these previous studies, Graham et al. (*18*) examined this phenomenon in the pink bollworm. As earlier cited, these investigators found that doses of radiation over 20 krad produced very few R_1 progeny, and all of these were sterile. As the dose of radiation decreased from 20 to 5 krad, the number and fertility of R_1 progeny increased.

Table 1 shows a brief summary of the data on number of progeny produced by various matings in these experiments by Graham et al. (*18*). In every case, reproduction was reduced in the second generation as compared with that of the first, whether or not the R_1 individuals were outcrossed to untreated males or inbred. Reproduction appears to have recovered somewhat when R_2 progeny were outbred to normal individuals. Fertility was higher among the R_2 generation progeny when the treated parent was a female than when the treated parent was a male. Sex ratios of R_1 and R_2 progeny were distorted, and males became more numerous as the radiation dose increased.

Cheng and North (*8*) examined this question further, using doses of 15 and 20 krad to the parental males. These investigators essentially confirmed the data of Graham et al. (*18*), indicating that, at both doses, R_1 male progeny were more sterile than R_1 female progeny, and all R_1 progeny were more sterile than the irradiated male parent. However, Cheng and North (*8*) extended their analysis to the relationship of sperm transfer in the female to oviposition. All of the males performed copulation (spermatophore transfer) well, but only about half of the treated males transferred eupyrene sperm. This result indicates an inability of the treated R_0 males or their R_1 progeny to physiologically satisfy the mated female. When treated males are caged with untreated females, an increase in number of transferred spermatophores leads to the same conclusion.

LaChance et al. (*24*) examined doses of radiation lower than previous investigators by carrying out an experiment with 2.5, 5.0, 7.5, 10, and 12.5 krad applied to males only. All essential conclusions of previous studies were confirmed by these investigators; however, they could find no effect of treatment on mating or eupyrene sperm transfer of any dose given. Normal females mated to

TABLE 1.--*Number of progeny produced by matings of irradiated males with normal females and by matings of* R_1 *and* R_2 *progeny*

[Data from Graham et al. (*18*)]

Dose given to parents (R_0)		R_1 progeny per female	Percent of control	R_1 mating		R_2 progeny per female	Percent of control	R_3 progeny per female	Percent of control
Male	Female			Male	Female				
Krad		*Number*				*Number*		*Number*	
0	0	77.4	100	0	0	27.4	100	47	100
5	0	44.8	57.8	R_1	0	.54	1.9	0	0
5	0	44.8	57.8	R_1	R_1	.02	.07	19.9[1]	42.3[1]
10	0	28.6	36.9	R_1	0	0	0	0	0
10	0	28.6	36.9	R_1	R_1	0	0	0	0
15	0	6.9	8.9	R_1	0	.18	.6	8.9	19.1
15	0	6.9	8.9	R_1	R_1	< .01	< .04	42.7[1]	90.8[1]
20	0	1.6	2.0	R_1	0	0	0	0	0
20	0	1.6	2.0	R_1	R_1	0	0	0	0

[1] R_2 X R_2 matings did not reproduce at these doses; these values are for R_2 males X untreated females.

the treated males tended to lay less eggs than the control females, and two doses (7.5 and 12.5 krad) were significantly lower than the control. Hatch of the eggs laid by these females was also significantly lower than hatch of control eggs at all doses but 5 krad. Sex ratio of the R_1 progeny was skewed in favor of males, significantly so at doses above 5 krad.

When LaChance et al. (*24*) looked at reproduction of R_1 individuals, they found that eupyrene sperm transfer decreased with increasing dose of radiation given to the parents. At 10 krad, only 78 percent of the R_1 males mated with normal females, and of those females that did mate, 47.2 percent contained only apyrene or no sperm. Thus, the R_1 males failed to successfully inseminate their mates and the females were physiologically unsatisfied. These authors do show that the failure to mate and transfer eupyrene sperm is a defect of R_1 males and not of R_1 females.

At doses of radiation above 5 krad, R_1 fertility was lower than R_0 fertility for the same dose. This was true for R_1 males or R_1 females mated to untreated mates or if the R_1 individuals were inbred. The authors concluded that R_1 males could be useful in a SIRM program for the pink bollworm even though the R_1 males showed some mating difficulties.

LaChance et al. (*27*) studied embryonic development of R_2 individuals produced by R_1 males crossed to normal females or R_1 females crossed to normal males. The doses studied were 6.5 and 13 krad. For both doses, R_1 individuals showed reduced egg hatch even though all eggs were produced by inseminated females. Embryological development was delayed in every case that an R_1 individual was mated to a normal individual; however, the R_2 embryos did not die at an early age but rather most developed to the hatching stage but did not hatch.

Berg and LaChance (*5*), using higher doses of radiation (19 and 30 krad), extended and confirmed the observations of the previous paper to directly treated sperm rather than to R_1 sperm. One significant conclusion of this paper is that the delay of treatment to adult males (48 to 72 hours postemergence as compared with 24 hours postemergence) resulted in an increase in mating ability and eupyrene sperm transfer even at a dosage of 30 krad. These authors also concluded that most of the effects of irradiation on egg hatch occur late in embryonic development.

RADIATION RELATED STUDIES

Radiation biology of the pink bollworm has been studied or referred to by many authors. Some of the more interesting results are covered here.

Haverty and Ware (*19*), studying circadian rhythms in laboratory populations of pink bollworms, found differential sensitivity of the moths to radiation depending upon the time of the light-dark (L-D) cycle in which they were treated. Sensitivity was measured by longevity of the treated adults. Both male and female moths were found to be significantly less sensitive to radiation one-half hour after lights were turned on than at any other time during a 24-hour period. Maximum sensitivity occurred 1-1/2 hours after lights were turned off. These results correlate very well with times of minimum and maximum activity. These results indicate that if rearing is done under a diurnal cycle, radiation treatments should be given shortly after lights on to achieve maximum longevity of the treated adults. Two recent studies by LaChance et al. (*28*, *29*) have examined

the diurnal rhythms of sperm production and will be discussed later. There have been no sequential studies of diurnal rhythm effects on induced sterility after irradiation. Such studies should be done.

Haverty and Ware (*19*) also found that mean longevity of adults decreased as the dosage rate increased. Five dosage rates (0, 1.0, 1.5, 2.0, and 2.5 krad/min) of X-irradiation were given for a total of 25 krad. The greatest change between dosage rates was between 1 krad and 1.5 krad. Females lived longer than males under all dose rates and times of irradiation.

In an article on the pink bollworm eradication efforts using radiation-sterilized pink bollworms, Knipling (*21*) suggested that the inability of irradiated moths to suppress native populations in the Coachella Valley of California was due to substantial movements of fertile moths from outside the suppression zone into the zone. His original proposal for the suppression tests suggested a release ratio of 50 sterile moths to 1 native moth. He also assumed that the released moths were only 25 percent efficient in competition with native moths. Trap records and subsequent analysis of migration potential of the pink bollworm demonstrated that such release ratios were never attained in the Coachella Valley and Borrego Springs. However, according to Knipling's analysis, the ratio of sterile to native moths in excess of 50:1 had been reached in the San Joaquin Valley by 1970, and no evidence of a self-perpetuating population of native moths has yet been demonstrated in that area.

LaChance et al. (*28*) have developed a scenario for the progression of eupyrene sperm bundles from the pink bollworm testes through the vas deferens, the seminal vesicles, and the duplex region of the male reproductive system. This movement shows a periodic rhythm associated with L-D cycles. During most of a 12:12 L-D cycle (the last three quarters of the cycle), sperm bundles descend from the testes and accumulate in the upper vas deferens. During the first 6 hours after the lights come on, these sperm bundles descend into the seminal vesicles. During the final 6 hours of light (in a 12:12 L-D cycle), the sperm bundles accumulate in the duplex region of the ejaculatory duct and are ejaculated from this region upon mating. A dose of 30 krad gamma-radiation appeared to have no significant effect on sperm bundle counts in young males. In older males, counts were reduced somewhat due to radiation.

About 10 to 20 sperm bundles moved into the duplex daily and were stored there until the male mated. This accumulation seemed to be fairly constant within a strain, but some variation existed between the strains the authors were examining.

The sequence of sperm bundle movement developed by LaChance et al. (*28*) was used by LaChance et al. (*29*) to study sperm susceptibility to radiation and also female ovipositional responses after mating with males that had been subjected to carefully timed radiation exposure (20 krad). Males were irradiated at exactly 9 hours after the onset of the light cycle of a 12:12 L-D regimen. According to LaChance et al. (*28*), the males would have eupyrene sperm bundles in the duplex region of the ejaculatory duct and in the testes, but other parts of the tract would be devoid of sperm. If a male mated during the next dark cycle, only sperm from the duplex region would be transferred. Control and irradiated males were placed with individual females for 24 hours, then transferred to new females. A total of four virgin females were sequentially presented to the males. The researchers (*29*) found that the first ejaculate by a 2- to 3-day-old male elicits the strongest ovipositional response in his mate. Second to fourth matings were very similar to each other but lower than the first mating for both control and

irradiated males, but irradiated males produce a lower ovipositional response than unirradiated males in any mating sequence.

A significant finding of this study (*30*) was that at 20 krad, no difference in egg hatch was found among matings. In other words, sperm in the testes and in the duplex region were equally sensitive to the effects of radiation.

Rush and Ware (*40*) conducted studies to determine whether radiation changed the susceptibility of pink bollworms to DDT, carbaryl, and azinphosmethyl insecticides.[6] They treated 6- and 7-day-old pupae with 10 krad of gamma-radiation and then topically treated the resulting adults with the three insecticides. Mortality was established 48 hours after exposure to the insecticide. Radiation exposure of the irradiated moths to carbaryl did not significantly alter their mortality curve, but the irradiated moths did show a slightly increased tolerance. Irradiated moths were significantly more susceptible to azinphosmethyl than unirradiated moths. The mode of action of azinphosmethyl appeared different in irradiated moths than in unirradiated ones. These authors attributed differences in response of the irradiated moths to different insecticides to the modes of detoxification used by the insect for each poison.

Wolfenbarger and Graham (*44*) conducted similar insecticide toxicity studies except that they irradiated 24-hour-old adults rather than pupae. In addition to carbaryl and azinphosmethyl, these authors tested methyl parathion, monocrotophos, and malathion.[7] The dose of irradiation used was 25 krad of gamma irradiation. This test differed in major ways from Rush and Ware (*40*): (1) The radiation dose was 25 krad rather than 10 krad; (2) the dose rate was 1.927 krad/min rather than 83 rad/min; (3) adults rather than pupae were irradiated; and (4) the strains differed significantly in that Rush and Ware used field-collected larvae whereas Wolfenbarger and Graham used a long-established laboratory strain. Wolfenbarger and Graham found no significant differences in response to the five insecticides of irradiated or unirradiated moths. The slope of the response line of irradiated adults treated with methyl parathion was much steeper (20.45) than for unirradiated moths (3.59), but the authors gave no statistical test for the difference. Changes in slope of a mortality response curve indicate changes or differences in mode of action of an insecticide.

The tests conducted by Wolfenbarger and Graham (*42*) on toxicity of insecticides for irradiated pink bollworm moths are closely related to present release conditions used by the USDA Animal and Plant Health Inspection Service in their SIRM program, but the differences seen by two groups of investigators indicate that caution must be exercised in extrapolation of these results. Tests should be run using appropriate insecticide choices, that is, those that are presently being used in pink bollworm control, and a dose and strain identical to the release conditions.

Several studies on the problems of handling insects for irradiation and shipment have been published, and the results are being used in the current SIRM

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[6]DDT = 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; carbaryl = 1-napthyl methylcarbamate; and azinphosmethyl = *O*,*O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate.

[7]Methyl parathion = *O*,*O*-dimethyl *O*-(*p*-nitrophenyl) phosphorothioate; monocrotophos = dimethyl phosphate ester with (*E*)-3-hydroxy-*N*-methylcrotonamide; and malathion = diethyl mercaptosuccinate S-ester with *O*,*O*-dimethyl phosphorodithioate.

program. (See Graham (*16*) and Richmond et al. (*36*, *37*)). Newly emerged adults can be held at temperatures between 1.7° and 4.5° C for up to 12 hours without showing a significant ill effect on mating or longevity. Adults can be irradiated under these conditions (which produce immobility) and shipped in a partially immobilized state, thus lessening damage due to the frenzied activity of tightly caged insects. Aerial drops of the insects utilize specially built refrigerated dispensers, which cause minimum damage to the insects (*20*). Using the assemblies described, a single pilot can dispense up to 3 million sterile moths without reloading.

FUTURE RESEARCH POSSIBILITIES

In spite of the volume of work done on pink bollworm radiation biology, there are still several unanswered questions. The most fundamental of these questions relate to the ability of released irradiated moths to control a field population. In two large release programs (Coachella Valley and Borrego Springs, California), no control was achieved. The lack of success in both programs could be attributed to: (1) The dispersive ability of pink bollworms, resulting in massive migration into the release zone; (2) low rates of sterile release as compared with the size of the resident native population, resulting in noneffective competition ratios of sterile to native moths; (3) loss of vigor or mating ability of released insects due to mass rearing, irradiation, handling, or disease; or (4) failure of the principle of SIRM. Most of the evidence cited by Knipling (*21*) favors the first alternative. Pink bollworm adults are strong flyers and have been found to appear on cotton up to 100 miles from the nearest infested field (Staten et al., unpublished data). In addition, the only evidence of noncompetitiveness in experimental tests is an increase in the number of spermatophores transferred by treated males to untreated females. This result indicates that such females are not effectively mated by the treated males and may continue to seek matings until a native male is encountered. If release ratios are high enough, such native times native encounters should be at a very minimum.

In practice, it may be very difficult to distinguish between alternatives 1 and 2; in principle, both factors can be corrected either by increasing releases or by decreasing the number of native insects with other control procedures.[8]

The sterile pink bollworm release program, begun in the San Joaquin Valley of California in 1969 by the USDA and the California State Department of Agriculture, still continues as of this writing. No self-perpetuating population of pink bollworms has been found in the San Joaquin Valley although large numbers of native males have been recovered in pheromone traps as part of the release program. The program must be regarded as a qualified success. The qualification is that no one is certain that the pink bollworm is adapted to the habitat found in the San Joaquin. Experiments are now being conducted to try to establish the biotic potential of the pink bollworm in the San Joaquin (R. T. Staten, personal communication) using radiation-sterilized diapausing larvae and partially sterilized (10 krad) adults. The results of these tests should help to define the efficacy of the releases now being made.

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[8]Since SIRM is most effective when used in conjunction with other control measures, the principles of integrated control should always be applied to any SIRM program.

A badly needed test of the pink bollworm SIRM is a field trial conducted in an area not subject to massive migration of native moths from outside the release zone but in which a continuous history of infestation is available. The three following areas seem appropriate for such a trial:

St. Croix, Virgin Islands

Populations of pink bollworms have been observed and measured on St. Croix; however, no commercially cultivated cotton is now present on the island. Populations are found only on escaped and wild cotton hosts. This location has the advantages of island isolation and low population densities. It has the disadvantages of distance from established rearing facilities, largely unknown past population densities, and geographically unique environmental conditions (as compared with areas in the United States where the pink bollworm occurs). Thus, results of a study on St. Croix would have to be extrapolated to commercial cotton-growing areas.

Baja California

R. T. Staten (personal communication) has conducted extensive surveys of pink bollworm populations in areas of Baja California. According to his analysis, the area of Mulege shows possibilities for a field trial of SIRM. Another possible area is La Ribera. In my opinion, Baja California has many of the same advantages and disadvantages as St. Croix. It is rather far removed from present rearing facilities and has the added disadvantage that none of the areas are really isolated from other cotton-growing areas so that immigration could be a problem. One advantage over St. Croix is that Baja California does have commercially grown cotton, and growing conditions could approximate the Arizona-California conditions.

Safford Valley, Ariz.

This area has recently experienced a significant reduction in conventional pink bollworm control efforts because of the Graham County pest management program. This program, utilizing pheromone traps and close population monitoring, has reduced populations to the point where by August 27, 1976, no insecticide treatments were needed for any of over 8,000 acres of cotton under the program (*1*). Pink bollworm populations were not eliminated but were held to noneconomic levels. Because of this pest management program, very good infestation records were available for every year since 1968. There is no evidence that significant migrations occur into the valley from other cotton-growing areas. The area is within easy flying distance of established rearing facilities, and expertise exists in the valley for monitoring of a release program. Environmental parameters are close enough to those of heavily infested areas for an experiment of this kind in a marginal area of pink bollworm growth.

When an organism is irradiated in an anoxic atmosphere (such as in nitrogen or helium), less genetic damage is induced than for a given radiation dose applied in air (*40*). This observation has led investigators to treat insects in anoxic atmospheres, hoping to increase competitive ability while maintaining high levels of sterility (*25*). Some positive effects of such treatments have been observed, especially in Dipterous insects, and could be investigated in the pink bollworm. One must keep in mind, however, that demonstration of protection of one physiological function (say, competitiveness) would probably indicate that other functions, such as sterility induction, would also be protected. Therefore, satisfactory experiments would have to include all such factors in their design. It would not be enough to demonstrate an effect of anoxia on mortality curves, flight ability, or ability to transfer eupyrene sperm, without also investigating level of sterility in similarly treated insects. With such strictures in mind, investigations of the protective effects of irradiation in anoxic atmosphere should be carried out on the pink bollworm.

Another report on modification of somatic effects of irradiation involves the use of the sex pheromone to increase genetic sensitivity to radiation while avoiding somatic damage. Calderon and Gonen (*7*) reasoned that exposure of male Lepidoptera to the female sex pheromone would increase oxygenation of the testes, thus increasing radiation sensitivity of the testes (this being the opposite effect of anoxia). Their data on *Cadra cautella* (=*Ephestia cautella*) showed a reduction of hatch from females mated to pheromone-treated irradiated males as compared with only irradiation-treated males. Such a result could easily be tested (and should be tested) in the pink bollworm. Such tests must be conducted in both mixed sex and single sex irradiation treatments.

Similar investigations could be made using deficiencies of divalent metal ions (for example, calcium and magnesium), which cause increases in chromosomal breakage in lower organisms. Involvement of major and trace elements in insect reproduction (*30*) suggests that manipulation of such ions in insect diets may have profound effect on radiation sensitivity in insects reared on such diets.

A possible approach to radiation sterilization of the pink bollworm, which has received little attention, is the use of neutrons. It is well known that neutrons are biologically more effective in inducing mutations than X- or γ-radiation. This is especially true when gross chromosomal rearrangements are considered. Low doses of neutron radiation could conceivably produce R_0 or R_1 sterility with little effect of the radiation on competitiveness. Preliminary results reported on *Galleria mellonella* (*41*) indicate that a mixed field of gamma and neutron radiation from californium 252 is more effective in inducing sterility than gamma irradiation alone. Such effects should be examined in the pink bollworm.

Another recent innovation in insect control is the use of laser beams. The absorption of a ruby laser pulse is proportional to the color of an insect. More energy is absorbed by darker pigments. Chromosomal damage can result from ruby laser exposure.[9] Since male pink bollworm larvae have dark purple testes, it

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[9]W. H. A. Wilde. Ruby and CO_2 lasers, their physiological and genetic effects on insects. *In* Symposium on Laser Applications in Entomological Research, Entomological Society of America Annual Meeting. Los Angeles. 1971. (Unpublished.)

might be possible to obtain a selective effect of laser exposure on the genetic material, thus producing chromosomal damage but little somatic damage. Several other research possibilities are suggested by the existence of mutations that alter pink bollworm larval coloration (Bartlett, unpublished data).

Fractionation of radiation dose over a period of time, rather than administration of an acute dose of radiation, has been examined in the confused flour beetle as a method to overcome some detrimental effects of radiation (*10*). This study demonstrated that these beetles can be sterilized, with little or no mortality, by suitable chosen fractionation patterns. The suitability of such an approach in a lepidopterous species is questionable, but since no such studies have been carried out in Lepidoptera, the technique should not be entirely dismissed.

L. E. LaChance (personal communication) has suggested that two serious gaps exist in our knowledge of pink bollworm radiation biology: (1) We have too little knowledge about sperm transfer after pupal irradiation at various doses, and (2) we know too little about the minimum sterilizing dose for females. At present, it seems highly impractical to sex the pink bollworm before release; thus, the need for more knowledge concerning female response to radiation.

This short discussion indicates only a few of the types of investigations that should be carried out in the pink bollworm to further our knowledge of its radiation response.

SUMMARY

An attempt has been made to summarize the effects of various doses of X-ray and gamma radiation on several stages of development of the pink bollworm in table 2. Several conclusions are apparent in looking at this table: (1) All doses studied in all stages had some effect on the treated insect or on their progeny; (2) lower doses (1 to 10 krad) have not been sufficiently examined, especially with regard to R_1 and later effects or with regard to effects in treated females; (3) early larval instars and older adults have been ignored; however, there may be valid program-related reasons for such neglect.

Another neglected area in the literature is the reporting of carefully designed competition experiments that could define the release ratios necessary to control a field population. Such studies are especially important in consideration of the use of delayed sterility (that is, use of partially sterilized R_0 individuals) because the released individuals will contribute to the field population, at least in the first generation. Data on the numbers of progeny to be expected from released partially sterile individuals could influence decisionmaking as to the feasibility of such releases.

The major detrimental effect of sterilizing doses of radiation on the pink bollworm appears to be in eupyrene sperm transfer, either from treated male to female or from the spermatophore to the spermatheca of the mated female. The observable effect of these difficulties is an increased propensity for mating of the female partner. This difficulty appears to be surmountable by increased release ratios. However, as more treated insects are needed for a given control level, costs of providing these insects increase proportionately. Therefore, research must be designed to see whether eupyrene sperm transfer can be improved

TABLE 2.--*Summary of radiation effects reported for the pink bollworm*[1]

Dose (krad)	Egg 1-2 days	Egg 3-4 days	Cutout larva	Pupa Early, 1-4 days	Pupa Late, 6-7 days	Adult 0-24 hr	Adult 48-72 hr
1	PS	F, PS					
2	H	PS	FE, R1FE R1S			R1PS	
4	H, PS, FD	H, PS,	FE, MPS, R1FE, R1S				
5-6		D, S, E	D, E	D, S		PS, R1PS	
8	H, PS, D	H, S, D L, E	D, E	D, S		PS, R1PS	
10		D, L, E	D, E	D, S	PS	PS, F, R1S,	
12		D, L, E		D, S		PS, F	
15		D, L, E	D, E	D, S	PS, F	PS, F, E, R1S	
20		D, L, E	D, E	D, S	S, F	S, F, E, R1S, R1E	S, good mating
25		D, L, E	D, E	D, S	S, F	S, E	
30		D, L, E	D, E	D, S	S, F	S, L	S, good mating
35				D, S	S, F, L	S, L	
40				D, S	S, F, L	S, L	
50				D, S	S, F, L	S, L	

[1]Key to radiation summary table; principle effects on a dose and stage are as follows:

H	An effect of the dose on hatching ability of the treated egg
PS	A partial effect of the dose on fertility of the treated adult or adults developing from the treated stage of development
S	Better than 90% sterility in treated adult or adult developing from treated stage of development
D	An effect of the dose on further development of a treated immature stage
L	An effect on the longevity of the treated adult or adult developing from a treated stage of development
M	Greater treatment effect on males rather than females
F	Greater treatment effect on females
R1	An effect on fertility of R_1 individuals
E	Lack of eupyrene sperm in mated females or no spermatophore was transferred (that is, no mating took place)

A blank indicates no reported results for a given dose and stage.

in the released insects. Cost effectiveness of any alteration in treatment conditions depends upon the ratio of costs to produce better insects versus the costs to produce more insects.

An area of research not covered by this review but of great concern to pink bollworm researchers is the behavior of the laboratory-reared released insects. H. M. Flint (personal communication) suggests that in any release program, the released insects compete at the level of finding the opposite sex at the right time. If laboratory insects do not compete at this level, eupyrene sperm transfer will not be a significant factor in the failure of a SIRM program. If SIRM programs are to be successful, we need information about released-insect behavior to use along with our knowledge of radiation biology. The success of mass-rearing programs, such as the pink bollworm program, has produced an increase in behavior research in recent years.

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